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Our initial proposal focused on developing technologies to uncover epigenetic changes that contribute to tumor development. Our initial attempts towards developing genome wide approaches to identify new genes silenced by epigenetic mechanisms encountered problems; however, our efforts to exploit epigenetic mechanisms of gene silencing tc study tumor suppressor gene function have been very successful (see below). Therefore, as we enter the second year of funding we plan to capitalize on the success of the latter experiments to refocus our effort. Our new objectives will build upon the unanticipated advances that we have made in the use of RNAi to manipulate gene expression in mouse and human cell systems and in animal models to explore the role of epigenetic modifications in breast cancer progression.

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Progress Report

Introduction

Our initial proposal focused on developing technologies to uncover epigenetic changes that contribute to tumor development. Our initial attempts towards developing genome wide approaches to identify new genes silenced by epigenetic mechanisms encountered problems; however, our efforts to exploit epigenetic mechanisms of gene silencing to study tumor suppressor gene function have been very successful (see below). Therefore, as we enter the third year of funding we plan to continue to capitalize on the success of the latter experiments to extend our refocused efforts.

Body
Key accomplishments

1.Control of Cellular Senescence. Cellular senescence is an extremely stable form of cell cycle arrest that limits the proliferation of damaged cells, including cells encountering telomere malfunction or DNA damage. As a consequence, mutations that disable senescence contribute to cellular immortalization and drug resistance in breast epithelial cells and other cell types. Work from our groups and others indicate that the p53 and p16/Rb tumor suppressor pathways are crucial regulators of senescence, but how activation of these pathways leads to a permanent arrest has remained largely unexplored. Previously, we identified and characterized the senescence associated heterochromatin foci (SAHFs) and proposed that epigenetic regulation of the senescence specific gene expression contributes to the "irreversibility" of the phenotype(Narita et al., 2003; Narita and Lowe, 2004). This year, to further characterize the heterochromatin components of senescence, we examined the profile of chromatin-binding proteins in HDFs by biochemical approach and identified the senescence specific chromatin binding proteins (Narita et al, in preparation). Among those we focused on HMGA2, since it was enriched in SAHFs. HMGA2 is a member of non-histone chromosomal proteins, which participate in a wide variety of cellular processes including transcription, chromatin organization, and cell cycle regulation. Although, HMGA2 expression has been linked to cell proliferation and tumor development, we paradoxically found that HMGA2 was upregulated in senescent cells and suppressed when cells were immortalized by E1A oncoprotein. Furthermore, overexpression of HMGA2 can induce SAHF-like chromatin condensation, p16 induction, and cell cycle arrest in a dose dependent manner. Finally, RNAi mediated down regulation of HMGA2 reduced SAHF formation and some senescent marker genes, such as p16 and stromelysin-1, in senescent HDFs. These results suggest that HMGA2 might be involved in the epigenetic regulation in tumor development/cellular senescence.

2. Analysis of the CBX7 oncogene. We are collaborating with David Beach to characterize the in vivo properties of the putative oncogene, CBX7. CBX7 is a member of the polycomb group (PcG) family that was identified by virtue of its ability to bypass senescence in prostate epithelial cells (Gil et al., 2003). Previous work indicates that Bmi-1, another PcG protein that silences the INK4a/ARF locus, is oncogenic when overexpressed in mice. Moreover, disruption of Bmi-1 leads to stem cell depletion, suggesting Bmi-1 can contribute to stem cell maintenance. To determine whether CBX7 has similar properties, we produced chimeric mice that expressed CBX7 in the hematopoietic compartment. We showed that CBX7 is a potent oncogene in vivo, capable of both initiating tumorigenesis and cooperating with c-myc to accelerate the onset of malignancies (Scott et al, in preparation). CBX7 is able to compensate for p53 loss in that the wt allele of p53 is retained during lymphomagenesis on an Em-myc p53 +/- background. The dependence of the oncogenic phenotype on INK4a/ARF is being addressed with lymphoma studies on an INK4a/ARF null background. Importantly, CBX7 does not co-localise with Bmi-1 and is thought to act in a different (Polycomb Repressive Complex 1) PRC1 complex. This is the first time that a PRC1 complex not containing Bmi-1 has been shown to be oncogenic. We are currently developing short hairpin RNAs (shRNAs) to suppress CBX7 function, and intend to use them to determine whether CBX7 acts as an oncogene by controlling the INK4a/ARF locus and/or influences stem cell maintenance. We are also generating mice in which the gene for CBX7 is ablated and will be able to compare the knock-down phenotype with the complete null (both conditional and germline). Since p16/INK4a is an important tumor suppressor in breast cancer, we hope these studies will help elucidate how epigenetic control of its expression can influence normal cell function and cancer development. Indeed, as CBX7 was cloned in a prostate screen, it may be that it has a role in the development of solid tumors, such as prostate cancer and breast cancer, rather than lymphoma, illustrating the utility of the lymphoma model to study oncogenic potential of candidate genes.

3. RNAi libraries and other new tools. Over the past year, we also have made advances in RNAi expression technology. For example, with funds from a variety of sources, we developed a large-scale resource for RNAi in mammalian cells (Paddison et al., in press). The initial library focused on covering the human genome and comprises some 30,000 sequence verified constructs. In addition, biochemical studies on the RNAi mechanism have allowed us to demonstrate that each expressed shRNA gives rise to a single, predictable siRNA. Using this information, we can now apply siRNA design rules to greatly increase the success rate for individual shRNAs. We are also exploring contextual requirements for shRNAs to gain entry into the RNAi pathway. In short, these studies have produced two critical insights. First, using design rules, the AVERAGE shRNA suppresses gene expression by more than 80%. Second, 29nt shRNAs are more potent (per mole of transfected RNA) than siRNAs at suppressing gene expression. Over the past year, we have constructed libraries of these constructs with the support of an Innovator award, and these are being integrated into this program as tools to study the epigenetics of tumor development.

Reportable outcomes

Papers published

Narita, M., Nunez, S., Heard, E., Narita, M., Lin A. W., Hearn, S. A., Spector, D. L., Hannon, G. J. and Lowe, S. W. (2003) Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 113: 703-716.

Narita, M. and Lowe, S. W. (2004) Executing Cell Senescence. Cell Cycle 3:244-246.

Conclusions

Over the last year, we have made substantial progress in our efforts to understand how epigenetic alterations can serve as tumor suppressor mechanisms. In the next year, we will continue to pursue the goal of understanding this connection.